

Macrostomida (Platyhelminthes: Rhabditophora) from Argentina, with Descriptions of Two New Species of *Macrostomum* and of Stylet Ultrastructure

Francisco Brusa*

*División Zoología Invertebrados. Museo de La Plata, Paseo del Bosque s/n°1900,
La Plata, Buenos Aires, Argentina*

In this work, two new species of the genus *Macrostomum* (*M. velastylum* n. sp. and *M. puntapiedrensis* n. sp.) are described from the littoral benthos of the Río de la Plata estuary, Argentina. This is the first description of members of the Macrostomidae from Argentina, and of a species documented from both limnic and brackish-water environments. *Macrostomum velastylum* differs from its congeners in the fine details of its stylet, which are described using a new technique for stylet isolation and observation (scanning electron microscope). This method provides new information on stylet structure relative to traditional whole-mount techniques. *Macrostomum puntapiedrensis* differs from others species of the *M. orthostylum* group in the length and morphology of the distal tip of the stylet. Further details are provided on additional macrostomids from Argentina, including potential conspecifics of *M. vej dovskyi*, *M. viride*, and *M. lineare*.

Key words: *Macrostomum velastylum* n. sp., *Macrostomum puntapiedrensis* n. sp., Río de la Plata, Argentina, Turbellaria, diversity, *Microstomum*

INTRODUCTION

Macrostomids are small- and medium-sized Rhabditophora (Platyhelminthes) known from around the world and occurring in a great diversity of environments, including limnic, brackish, and marine (e.g., Ferguson, 1939a-e, 1940a-c, 1954; Noreña *et al.*, 2003). Of the approximately 200 known species of Macrostomida (Tyler *et al.*, 2005), only 29 are described from South America. Among them, the greatest number of species has been reported for Brazil (16 species), and the majority of these are marine (11 species), with some present in brackish and limnic environments (Marcus, 1946, 1949, 1950, 1952, 1954; Gamo and Leal-Zanchet, 2004). *Macrostomum hystricinum* Beklemishev, 1951 and *Macrostomum phocurum* Marcus, 1954 are the only two species described from brackish environments along the South American coasts.

Thirteen marine species from the Galapagos Islands are also known (Ax and Schmidt, 1973; Sopott-Ehlers and Schmidt, 1974a, b; Schmidt and Sopott-Ehlers, 1976), and a dubious marine species has been cited for Venezuela (Hyman, 1955). In Argentina, only *Microstomum lineare* (Müller, 1774) O. Schmidt, 1848 has been reported, from the middle Paraná River (Noreña-Janssen, 1995).

In this work, two new species of the genus *Macrostomum*, *M. velastylum* n. sp. and *M. puntapiedrensis* n. sp., are described. These represent the first report of macrostomids from Argentina, and of a species from both limnic and

brackish environments. Furthermore this is the first time that specimens comparable to *Macrostomum vej dovskyi* Ferguson, 1940 and *Macrostomum viride* Van Beneden, 1870 have been reported from South America.

MATERIALS AND METHODS

Samples were taken seasonally from August 2000 to January 2004 at two sampling sites in the littoral of the Río de la Plata, Argentina. The localities were Atalaya (35°00'53.6"S, 57°32'3.3"W) and Punta Piedras (35°21'23.1"S, 57°10'22.5"W) in Buenos Aires Province (Fig. 1). In both localities, samples were taken of the littoral benthos of the river and of the floating vegetation in environments associated with the river (Tables 1, 2). These environments at the Atalaya locality comprised the littoral zone of the river in a region covered with a great quantity of floating vegetation, and at the Punta Piedras locality comprised a lotic environment (La Matilde Stream).

Samples of benthos were taken with corer tubes 4.5 centimetres in diameter, along a transect from the upper tidal level (UTL) to the lower tidal level (LTL). The first two centimeters of sediment were taken to the laboratory to be observed *in vivo*. Samples from the vegetation zones were taken using hand nets with an 800 cm² opening and 40–80 µm mesh size. All samples were transported *in vivo* to the laboratory in plastic bags.

The environmental parameters measured were salinity, conductivity, TDS, pH, dissolved O₂, O₂ saturation, and water temperature (Tables 1, 2). Substrate type and associated fauna were also recorded (Tables 1, 2).

In the laboratory, microturbellarians were separated and observed *in vivo* as whole squash-mounts. They were then fixed in Bouin's solution, serially sectioned in the sagittal plane every 4 µm, stained with the Azan method, and mounted in synthetic Canada balsam. Other specimens were whole-mounted in polyvinyl-lactophenol to study the male stylet. Some specimens were dissected; their stylets were extracted, air-dried, and coated for observation in

* Corresponding author. Phone: +54-221-4257744;
Fax : +54-221-4257527;
E-mail: fbrusa@fcnym.unlp.edu.ar

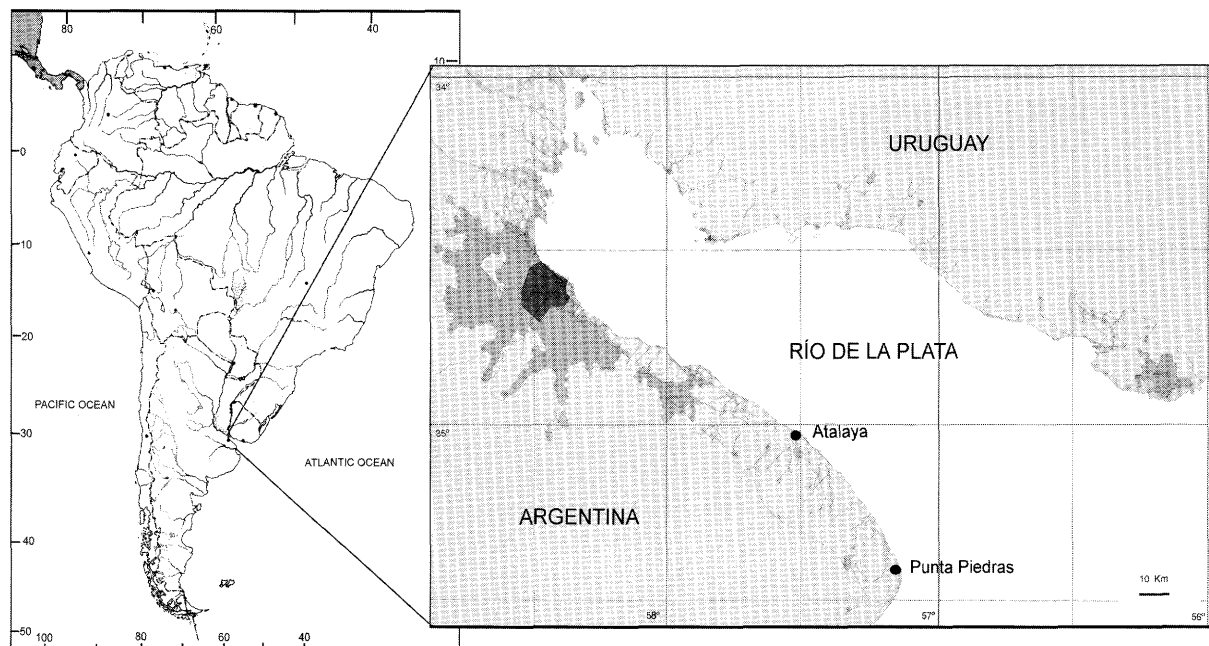


Fig. 1. Map of South America showing the location of the study area; inset shows the locations of sampling sites.

Table 1. Sample date, environmental data, substrate type and associated fauna for Punta Piedras.

	salinity (‰)	conductivity (μ S)	TDS (mg/l)	pH	O ₂ (mg/l)	O ₂ sat. (%)	water T° (°C)	type of substrate	associated fauna
Punta Piedras									
2000.8.2	#	2750	1350	#	#	#	13	Caliche-clay/muddy*	#
12/02/2001**	2.5	1171	587	7.94	#	#	27.5	Caliche-clay/muddy*	#
2001.8.24	4	2110	#	7.89	10.05	116	20.7	Caliche-clay/muddy*	#
05/12/2001**	5	9650	4790	9.46	10.22	142	33.1	Caliche-clay/muddy*	polichaeta, nematoda, gastropoda
2002.4.4	4	5080	2600	8.9	5.5	100	27	Caliche-clay/muddy*	#
2003.3.5	1.5	757	368	7.63	8.8	115	28.7	Caliche-clay/muddy*	acari, copepoda, cladocera, ostracoda, nematoda, oligochaeta

without data.

* from Darrigran (1999).

** dates in which turbelarians were found.

a JEOL 100 scanning electron microscope (SEM).

The material has been deposited in the Invertebrate Zoology Collection of the Museo de La Plata, Argentina (MLP).

RESULTS

Family Macrostromidae

Genus *Macrostromum* O. Schmidt, 1848

Macrostromum velastylum n. sp.

Figs. 2–4

Material examined. Holotype (MLP 5554), specimen mounted in polyvinyl-lactophenol, from pond near littoral of river in Atalaya, 2 August 2001. Paratype, six specimens (MLP 5333 and 5334), serial sections cut in the sagittal plane, same locality as holotype, 2 February 2002, and La Matilde stream, 19 April 2003. Other material, a dried male stylet observed by SEM, Punta Piedras benthos, 12 February 2001.

Etymology. The specific name derives from the Latin *vela* (veil), referring to the presence of lateral projections

(veils) on the male stylet.

Description. Anterior end rounded, body thin. Posterior end truncate, with slight sub-terminal constriction. Live squash-mounted specimens 1.68 mm long by 0.48 mm wide. Cilia distributed homogeneously over whole body surface; long and rigid cilia at the anterior and posterior ends. Rhabdites abundant in the epidermis, arranged in groups of 2 or 3, more abundant in posterior end of the body (Fig. 4D), scarce on ventral surface between mouth and male gonopore. Body wall between male and female gonopore with longitudinal musculature better developed than in rest of body.

Pair of small eyes associated with posterior region of brain, at level of anterior end of pharynx (Fig. 2B). Small, ciliated pharynx leads to broad intestine which extends up to anterior region of male stylet.

Male reproductive system with pair of testes situated at sides of anterior region of intestine (Figs. 2B, 4F). Each vas deferens enters piriform seminal vesicle with very thick muscular walls. Seminal vesicle continues into a thin muscular

Table 2. Sample date, environmental data and vegetation type for La Matilde stream and Atalaya localities.

	salinity (‰)	conductivity (μ S)	TDS (mg/l)	pH	O ₂ (mg/l)	O ₂ sat. (%)	water T° (°C)	type of vegetation
La Matilde								
12/02/2001**	#	#	#	#	#	#	#	<i>Lemnaceae</i>
2001.3.5	0	279	142	6.06	#	#	26	<i>Lemnaceae</i>
2001.8.24	1.5	387	#	6.4	4.13	42.9	16.3	#
2001.12.5	0	1439	725	7.7	1.9	23.2	24.7	few <i>Lemnaceae</i> , in decomposition
2003.3.5	0.5	1813	918	7.14	0.28	3.2	28.4	A lot of <i>Lemnaceae</i> , in decomposition
19/04/2003**	#	1171	580	7.02	0.48	4.6	16.5	<i>Lemna giba</i>
Atalaya								
2000.3.31	#	#	#	#	#	#	#	<i>Pistia stratiotes</i> and <i>Lemnaceae</i>
2001.3.5	0	358	181	5.73	#	#	26	<i>Salvinia biloba</i> and <i>Limnobium spongia laevigatum</i>
02/08/2001**	0	879	441	6.28	0.28	2.4	11	<i>Pistia stratiotes</i> , <i>Limnobium spongia laevigatum</i> , <i>Salvinia biloba</i> and <i>Lemnaceae</i>
02/02/2002**	0	571	278	6.25	#	#	24	<i>Pistia stratiotes</i> , <i>Salvinia biloba</i> and <i>Hydrocotyle ranunculoides</i>
2002.4.4	1	1266	629	6.7	0.1	1	16.8	<i>Pistia stratiotes</i> , <i>Salvinia biloba</i> and <i>Hydrocotyle ranunculoides</i>
2002.6.25	#	501	278	7.05	#	#	5.8	<i>Salvinia biloba</i> and <i>Hydrocotyle ranunculoides</i>
2003.2.21	#	397	180	6.5	1.1	11.5	17.6	<i>Pistia stratiotes</i> and <i>Salvinia biloba</i>
09/04/2003**	1.5	438	220	7.03	6.19	62.2	16.7	<i>Pistia stratiotes</i>
2004.1.27	2	5490*	2750*	6.81	0.1	2	29.2	<i>Pistia stratiotes</i> and <i>Salvinia biloba</i> *

without data.

* The communication between the sample place and the Rio de la Plata was modified during the study. This provoked changes in water and vegetation quality.

** dates in which turbellarians were found.

duct to enter a broad prostatic vesicle, which has thick muscular walls; vesicle connected with stylet by a muscular ejaculatory duct. Stylet located in male atrium, the latter leading to mid-ventral male gonopore, posterior to female gonopore. Stylet tubular, tapering, with proximal end slightly broader than distal end, 155 μ m long, "C"-shaped, with three lamellae. One lamella (Fig. 3A, B, D: arrow a) extends from distal end to medial sector of stylet. Second lamella longer than the first (Fig. 3: arrow b), originates on side opposite to anterior lamella and follows a slightly helicoidal path, ending on concave side of stylet. Third lamella (Fig. 3A, C: arrow c) smallest, occupying middle region of stylet. Stylet opening oval, subterminal, located on convex side.

Female reproductive system with pair of ovaries lateral to intestine (Figs. 2, 4). Proximal region of each ovary with oocytes in first stage of development, maturing towards female gonopore. Oviduct communicates with female atrium by a sphincter. Female atrium, with thick, muscular walls and a well-developed ciliated epithelium, surrounded externally by a great number of small, unicellular glands. Ciliated vagina arises from atrium, surrounded by numerous cement glands that lead to female gonopore.

Testate amoebae (*Arcella* sp.), rotifers, and diatoms were found in the intestine.

Remarks. The presence of lamellae on the stylet is not a common feature among species of the genus *Macrostromum*. Likewise, in species where lamellae are present, their development is different from that observed in *M. velastylum* n. sp. The species most similar to *M. velastylum* n. sp. are *M. saifunicum* Nasonov, 1929 (reported only from Lake Biwa in Japan) and *M. inductum* Kolasa, 1971 (reported only

from Poznan, Poland). The stylets of these species, though similar in general appearance to the stylet of *M. velastylum* n. sp., form an extended cone with a distal curve. In *M. saifunicum*, the distal opening is terminal and elliptical, and the proximal end is obliquely truncate; one thin lamella is present in the distal fold, and the total length of the stylet is 130 μ m (Nasonov, 1929). In *M. inductum*, even though the opening is sub-terminal, the stylet ends in a "denticle"; it has a thin lamella, developed only in the distal curvature, and the total length of the stylet is 90–120 μ m.

Both *M. saifunicum* and *M. inductum* also possess a division in the seminal vesicle (Nasonov, 1929; Kolasa, 1971); this feature is not present in the new species described herein. Furthermore, specimens of *M. saifunicum* are slightly longer (2–2.5 mm) than those of the species described here (1.68 mm), and those of *M. inductum* are shorter (1.2–1.3 mm).

Both *M. saifunicum* and *M. inductum* have been described from limnic environments, whereas *M. velastylum* n. sp. was found in both limnic environments among floating vegetation and a brackish environment, indicating a high tolerance for a variety of environmental conditions (Tables 1, 2).

Macrostromum puntapiedrensis n. sp.

Figs. 5–6

Material examined. Holotype (MLP 5354), serial sections cut in the sagittal plane of one specimen, Punta Piedras benthos, 5 December 2001. Paratype (MLP 5555), serial sections cut in the sagittal plane of two specimens, Punta Piedras benthos, 5 December 2001.

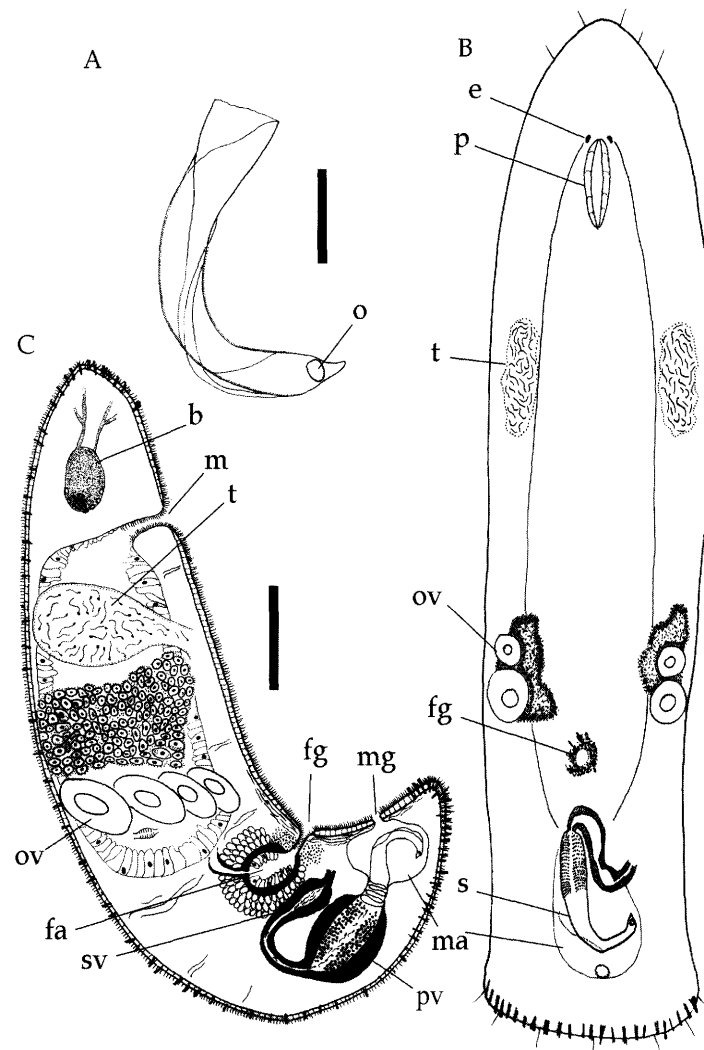


Fig. 2. *Macrostomum velastylum* n. sp. **A)** Stylet; **B)** ventral view; **C)** sagittal reconstruction. Abbreviations: b, brain; cp, ciliated pits; e, eye; fa, female atrium; fg, female gonopore; i, intestine; m, mouth; ma, male atrium; mg, male gonopore; o, opening; ov, ovary; p, pharynx; pv, prostatic vesicle; rc, rigid cilia; rh, rhabdite; s, stylet; sp, sphincter; sv, seminal vesicle; t, testis. Scale A, 50 µm; scales B, C, 200 µm.

Etymology. The specific name refers to the name of the type locality, Punta Piedras.

Description. Length of mature specimens *in vivo*, 500–600 µm. Body digitiform, the ratio between length and width smaller than in other species of the genus studied here, giving it a more robust aspect. Cilia cover the body uniformly, and in anterior and posterior regions, there are scarce longer and rigid cilia. Eyes present at anterior region of pharynx (Figs. 5B, 6). Rhabdites in groups and generally of large size (Fig. 6B), particularly in posterior region of body.

Male reproductive system consists of pair of testes in anterior half of body, at sides of the intestine and behind pharyngeal region. Seminal vesicle, in live specimens, situated behind region of female gonopore, oval in shape, its proximal region oriented towards posterior end of body and distal region towards anterior end. Vesicle opens into narrow duct that, before ending stylet, has a strongly muscular zone, the prostatic vesicle. Tubular stylet long (119 µm), slightly broader at base and gradually thinner towards distal end. Male gonopore very near posterior end of body.

Remarks. The tubular stylet is long (119 µm), slightly

broader at the base and gradually thinner towards the distal end, the distal tip without thickened walls (the *Macrostomum orthostylum* group). These are the diagnostic characteristics of *M. puntapiedrensis* n. sp. Within this group of species, *Macrostomum orthostylum* Braun, 1885 presents a stylet that is similar but shorter, 61 µm long (in Finland specimens) and more pointed (Luther, 1960) than that of the new species. Luther (1960), in his revision Scandinavian specimens, mentions that the specimens vary in length from 1,200 µm in Finland to 1,700–2,400 µm in Austria and Switzerland. These sizes are greater than those of the specimens studied here (500–600 µm). Another difference is that *M. orthostylum* has a muscular seminal vesicle and a short intervesicular duct. In *M. puntapiedrensis* n. sp., the seminal vesicle is oval in shape, without a muscular wall. The vesicle opens into a narrow duct that, before ending in the stylet, has a strongly muscular zone, the prostatic vesicle.

Macrostomum cf. *M. vej dovskyi* Ferguson, 1940

Fig. 7

Material examined. One squash-mounted specimen *in vivo*, associated with floating vegetation in La Matilde

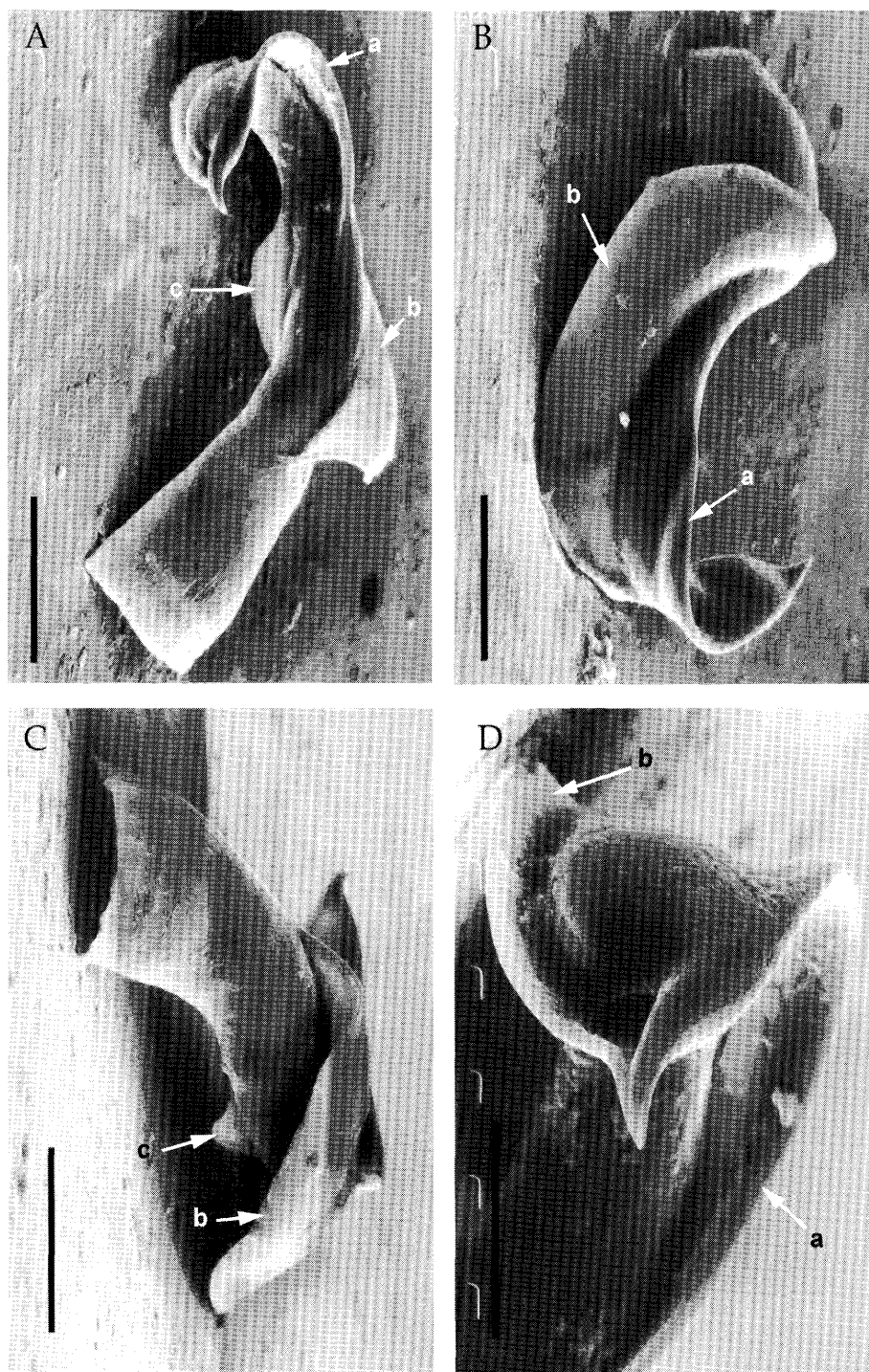


Fig. 3. *Macrostromum velastylum* n. sp. Scanning electron micrographs (SEM) of the stylet. **A–B**) General view; **C**) detail of the small veil; **D**) detail of the distal end. Arrows show the lamellae (see text for details). Scales A–C, 40 μ m; scale D, 2 μ m.

Stream (12 February 2001).

Description. Specimen digitiform, with a slight widening of posterior end of body where it attaches to substrate. Surface of body covered by cilia, some of which are longer and more rigid at anterior and posterior ends of body. Rhabdites arranged in groups of 2–3, more numerous in posterior end of body. Ovaries present at sides of intestine in posterior half of body, with asymmetric development, one of them larger. Female gonopore surrounded by glandular cells. Stylet (52 μ m long), the only structure observed from male reproductive system, is short, conical, with wide, rapidly

tapering base. Base region more robust in appearance than rest of the stylet; distal end beveled.

Remarks. The stylet, which is a specific diagnostic feature for *Macrostromum* species, is 52 μ m long, has a conical shape with a basal region slightly widened and the apical zone ending in a bevel. These features coincide almost completely with those mentioned by Ferguson (1940b) for *M. vej dovskyi* Ferguson, 1940: tubular curved stylet with the proximal end somewhat widened and crenate, the opening subterminal, and distal part tapering. The length of the stylet in *M. vej dovskyi* (Ferguson, 1940b) is smaller (35 μ m long)

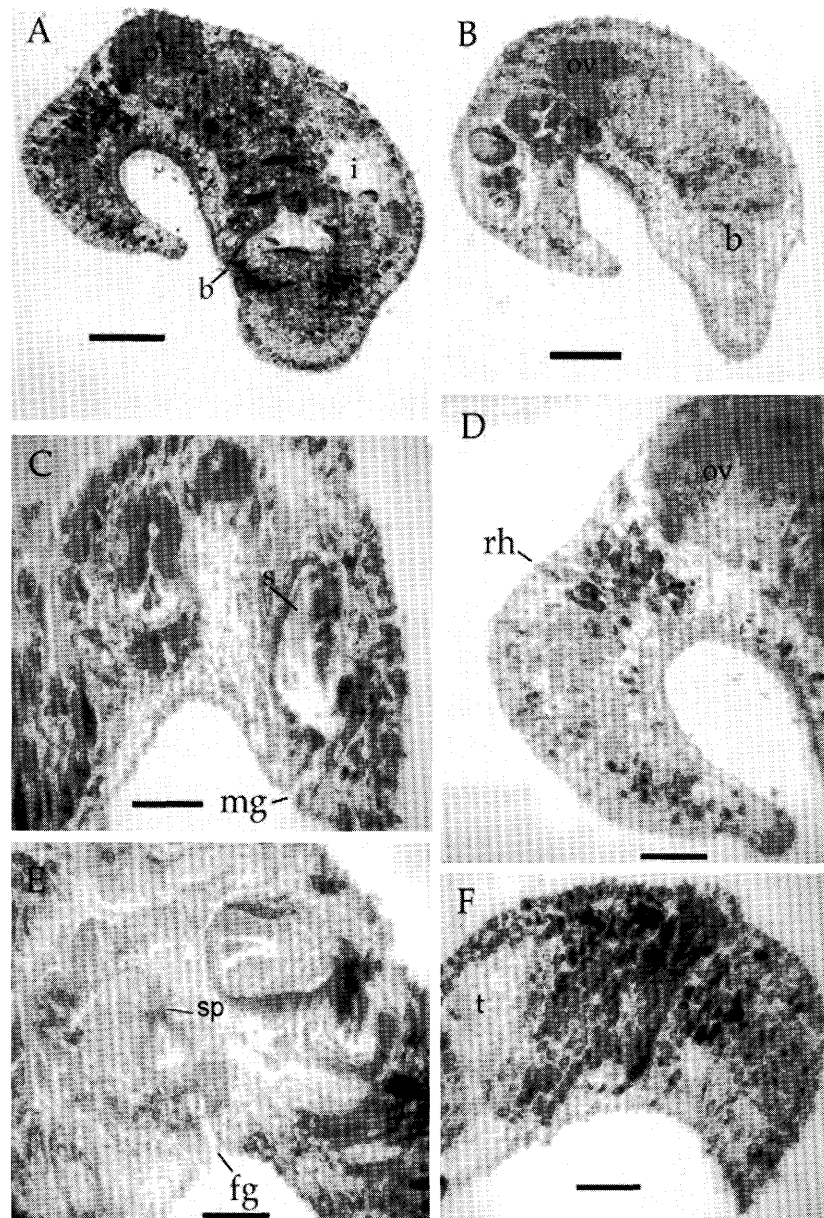


Fig. 4. *Macrostomum velastylum* n. sp. Sagittal sections. **A)** General view, ovary and intestine; **B)** general view, male and female reproductive systems; **C)** detail of the posterior region of the body showing male and female reproductive systems; **D)** detail of the posterior region of the body showing ovary and epidermis with rhabdites; **E)** detail of the female gonopore; **F)** medium region of the body showing testis. See Fig. 2 caption for abbreviations. Scales A, B, 100 μ m; Scales C–F, 50 μ m.

than that of the specimen found here.

Distribution. *M. vejovskyi* is previously known from New Haven, Connecticut, USA (Ferguson, 1940b).

Macrostomum cf. *M. viride* Van Beneden, 1870

Fig. 8

Material examined. Squash-mounted specimens *in vivo*, found associated with floating vegetation at Atalaya (2 February 2002).

Description. Body digitiform, anterior and posterior ends slightly thinner. Body covered with cilia, some more rigid, longer, and present at both ends of body; others distributed over entire surface of body. Pharynx small; eyes small, located at sides and in front of pharynx. Testes not observed. Small seminal vesicle located behind female gonopore, curving backwards to lead to stylet. Stylet (37 μ m

long) sigmoid in shape with proximal end widened; distal opening terminal, located in concave portion of stylet.

Pair of ovaries lateral to intestine, asymmetrically developed. Oocytes very large, deforming and widening the body in this region.

Remarks. Due to their scarcity and immature reproductive condition, specimens could not be reliably identified to species. However, the morphology of the stylet is similar to that of *Macrostomum viride*, though the latter has the distal end more pointed than in the specimens found here (Ferguson, 1940b). *Macrostomum finlandensis* (Ferguson 1940) Luther, 1947 has a sigmoid-shaped stylet (Luther, 1947), similar to that of the specimens studied here, though a little longer.

Distribution. *Macrostomum viride* is previously known from Europe (Ferguson, 1940b).

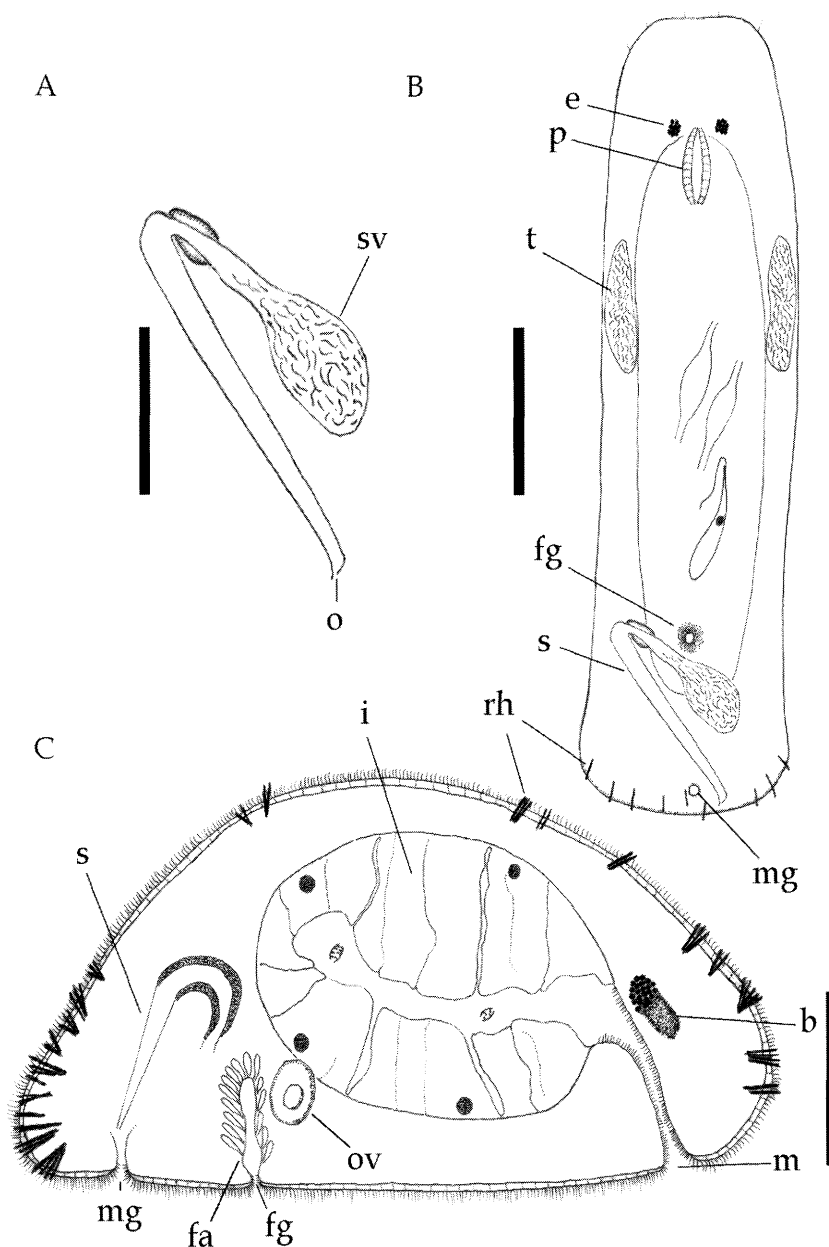


Fig. 5. *Macrostomum puntapiedrensis* n. sp. **A)** Stylet; **B)** ventral view; **C)** sagittal reconstruction. See Fig. 2 caption for abbreviations. Scale A, 50 μ m; scales B, C, 100 μ m.

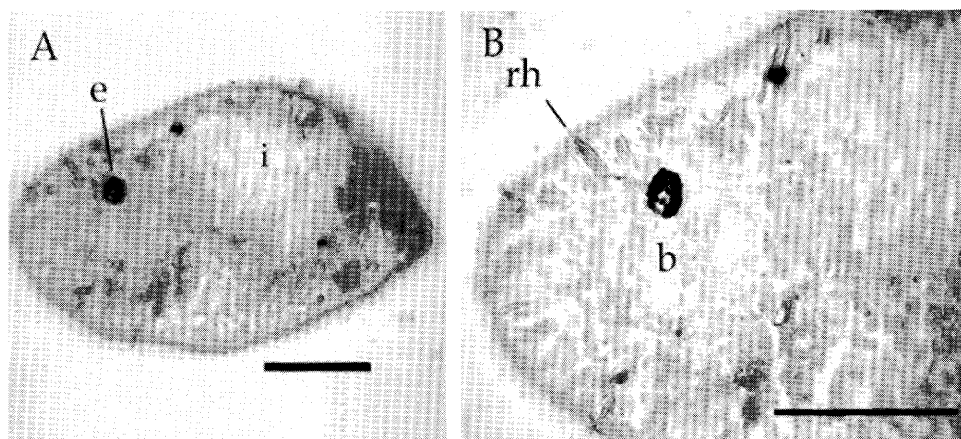


Fig. 6. *Macrostomum puntapiedrensis* n. sp. Sagittal sections. **A)** General view of the body; **B)** detail of the anterior region of the body showing rhabdites, brain, and eye. See Fig. 2 caption for abbreviations. Scale 50 μ m.

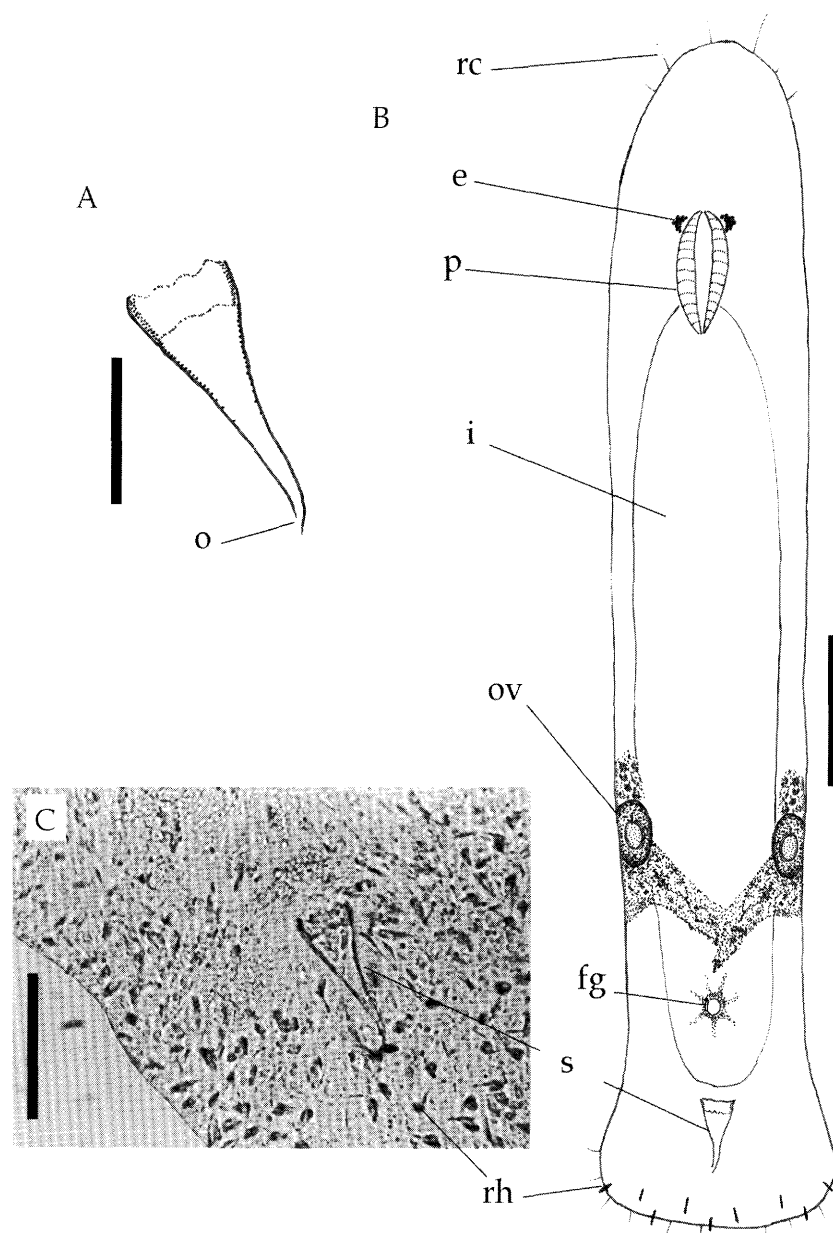


Fig. 7. *Macrostomum* cf. *M. vej dovski*. **A)** Stylet; **B)** ventral view; **C)** photograph of a specimen *in vivo* showing detail of the stylet. See Fig. 2 caption for abbreviations. Scale A, 25 μ m; scale B, 130 μ m; scale C, 50 μ m.

Family Microstomidae

Genus *Macrostomum* O. Schmidt, 1848

Macrostomum cf. *M. lineare* (Müller, 1774) O. Schmidt, 1848

Fig. 9

Material examined. Squash-mounted specimens *in vivo*, found associated with floating vegetation at Atalaya (9 April 2003) (Table 2).

Description. Specimens 0.5–5 mm long, depending on number of zooids forming body. Body tapering, anterior and posterior ends rounded. Color yellowish white. Anterior region of body with pair of lateral ciliated sensory pits and four brown pigmented epidermal spots. Body covered with abundant cilia. Mouth situated in anteroventral region of body, leading to simple pharynx that can be broadly protracted at moment of prey capture. Intestine, with a preoral caecum, continuous in the chain of zooids, forming expansions in constrictions of body. Cnidocysts were found in the

epidermis of some specimens.

Remarks. Body size is highly variable, depending on the number of zooids that form it, very similarly to what Noreña-Janssen (1995) mentioned for specimens from a lentic environment in the middle Paraná River, Argentina. As with Noreña-Janssen (1995), sexually mature specimens were not found. Microstomids can only be reliably identified if the stylet is known. For the purposes of the present study, the specimens found in this work are considered comparable to *Macrostomum lineare*. Some authors have attributed cnidocysts in the epidermis (cleptocnidia) to the ingestion of cnidarians (Karling, 1966; Noreña-Janssen, 1995).

Distribution. *M. lineare* is a cosmopolitan species. In South America, it has been reported only from Argentina, from the floodplain of the middle Paraná River (Noreña-Janssen, 1995).

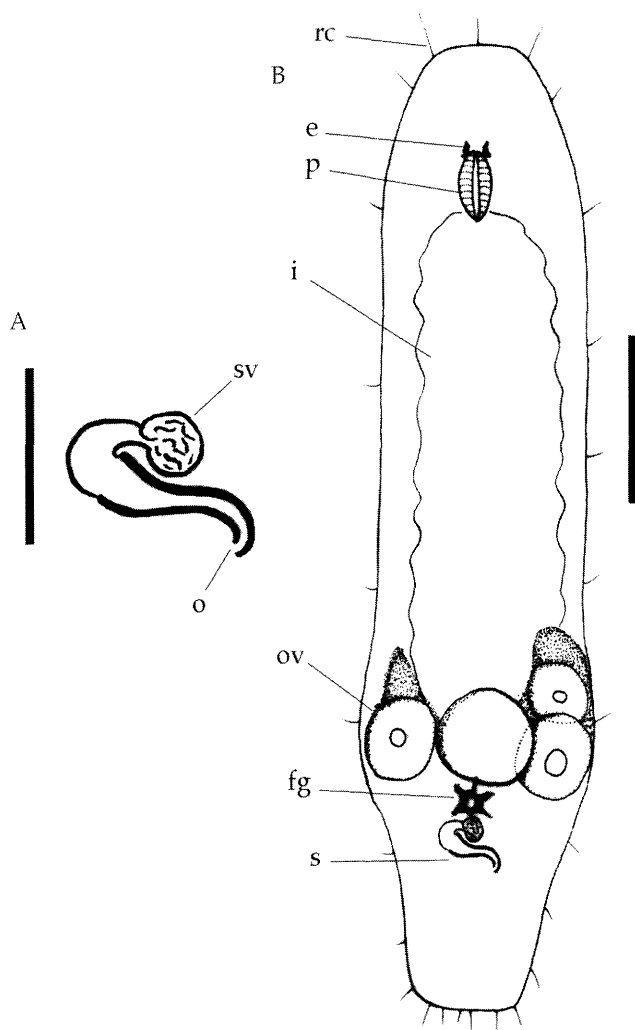


Fig. 8. *Macrostomum* cf. *M. viride*. **A)** Stylet; **B)** ventral view. See Fig. 2 caption for abbreviations. Scale A, 40 μ m; scale B, 180 μ m.

GENERAL REMARKS

All of the specimens assigned in this work to the genus *Macrostomum* are, with little doubt, new to science, but the specimens found in most cases did not allow adequate taxonomic resolution (except for *M. velastylum* n. sp. and *M. puntapiedrensis* n. sp.). Thus, most specimens were assigned to the known species most similar in morphology. These assignments are considered tentative until more complete descriptions can be made.

Of the approximately 200 known species of Macrostomida known worldwide, to date only 29 have been described from South America. Previous studies have revealed significant diversity in Brazil (Marcus, 1946, 1949, 1950, 1952, 1954) and the Galapagos Islands in Ecuador (Ax and Schmidt, 1973; Sopott-Ehlers and Schmidt, 1974a, b; Schmidt and Sopott-Ehlers, 1976). The current study reveals that the known diversity of macrostomids in South America is likely to increase with greater sampling and attention to the vast range of aquatic habitats, both coastal and inland, in Argentina.

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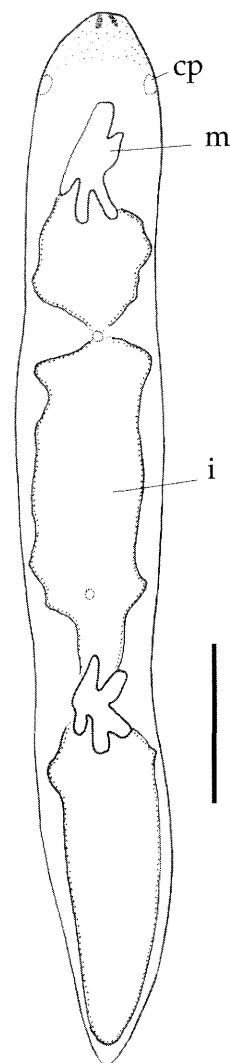


Fig. 9. *Macrostomum* cf. *M. lineare*, ventral view. See Fig. 2 caption for abbreviations. Scale, 200 μ m.

Carolina Noreña, who co-directed my PhD research. This study was supported by a doctoral fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

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